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לשימוש הלשכה
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בקשה לפטנט
Application For Patent

אני, (שם המבקש, מענו ולגבי גוף מאוגד – מקום ההתאגדות)
I (Name and address of applicant, and in case of body corporate-place of incorporation

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בעל אמצאה מכח הדין והעברה שמה הוא By law and assignment

Owner, by virtue of the

of an invention, the title of which is

(בעברית) שילוב מנגנוני פעולה בטיפול משופר בסרטן
(Hebrew)

(באנגלית) COMBINED MODALITIES FOR IMPROVED CANCER TREATMENT
(English)

מבקש בזאת כי ינתן לי עליה פטנט hereby apply for a patent to be granted to me in respect thereof.

*בקשה חלוקה- Division of Application	*בקשה פטנט מוסף- Addition Application for Patent	* דרישה דין קדימה Priority Claim		
מבקשת פטנט Application from	לבקשה/לפטנט to Patent/Application	מספר / סימן Number / Mark	תאריך Date	מדינת האגוד Convention Country
No.....מס	No.....מס			
dated.....מיום	dated.....מיום			
* יפוי כח: / מיוחד – / עוד יוגש P.O.A.: individual / to be filed later - filed in case _____ הוגש בעניין				

תיקנו: COLL/016 IL

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For the Applicant,

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Patent Attorney

היום 31 בחודש דצמבר שנת 2001
This 31 of December of the year 2001

לשימוש הלשכה
For Office Use

טופס זה כשהוא מוטבע בחותם לשכת הפטנטים ומושלם בספר ובתאריך ההגשה, הנו אישור להגשת הבקשה שפרטיה רשומים לעיל.
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שילוב מנגנוני פעולה בטיפול משופר בסרטן

COMBINED MODALITIES FOR IMPROVED CANCER TREATMENT

COLL/016 IL

COMBINED MODALITIES FOR IMPROVED CANCER TREATMENT

FIELD OF THE INVENTION

The present invention relates to the field of cancer treatment, specifically to the synergistic effects of administration of quinazolinone derivatives, especially halofuginone, together with other anti tumor treatments.

BACKGROUND OF THE INVENTION

Fibrosis

Clinical conditions and disorders associated with primary or secondary fibrosis are distinguished by excessive production of connective tissue, resulting in destruction of normal tissue architecture and function. Fibrosis results from diverse modes of trauma including burns, surgery, infection, alcohol and other types of toxins.

Acute fibrosis occurs as a common response to various forms of trauma including radiation and chemotherapy treatments.

Radiation and fibrosis

Radiation damage to normal tissues has gained in interest over the past few years both in experimental clinical oncology and fundamental radiobiology, because of the discovery that such damage could be reversible.

Radiation fibrosis is an extremely severe adverse effect without preventive or curative treatment caused by ionizing radiation. Fibrosis disorders has been described in almost any tissue, including skin, lung, heart, liver and kidneys.

No medical treatments have ever shown beneficial effect in treating acute complications (e.g., bowel obstruction). Surgery is rarely successful, and generally results in repeated operations with poor recovery.

Fibrosis is a sequel of both radiotherapy and accidental overexposures. the clinical conditions and disorders are distinguished by excessive production of connective tissue, resulting in destruction of normal tissue architecture and function.

Although radiation fibrosis has been reported for many years in histopathological studies, the mechanisms of its initiation and chronic extension still remain to be resolved. Fibrosis is in fact a dynamic process, characterized by a constant remodeling and long term fibroblast activation. In normal wound healing, fibroblasts are transiently activated into myofibroblasts to proliferate and deposit the collagen matrix. Feedback mechanisms then occur to down regulate cellular activities. It has been proposed that myofibroblasts are terminally differentiated cells that finally disappear by apoptosis. In fibrosis, on the contrary, the feedback regulations are not observed, and chronic, long term myofibroblasts activation is sustained. One possible origin of the chronic cellular activation could be an abnormal production of stimulating factors such as cytokines and growth factors.

An alternative new concept recently emerged concerning the initiation of radiation damage which proposed that a cascade of cytokines is initiated immediately after irradiation persists for long periods of time and leads to the development of late damage.

Chemotherapy and fibrosis

Some cytotoxic agents are known to induce fibrosis in different organs. One of the agents is Bleomycin, which is known to induce lung fibrosis. Although there are reports of pulmonary fibrosis with almost all chemotherapeutics agents, Bleomycin is certainly the most widely reported. Other agents associated with the highest incidence include busulfan, carmustine (BCNU), and mitomycin-C.

Bleomycin, the classic chemotherapeutic agent inducing pulmonary fibrosis, is reported to induce pulmonary fibrosis in approximately 10% to 30% of treated patients, with death cause by pulmonary fibrosis in 1% to 2% of patients (Wesselius L., J. Comp. Ther. 1999;25 (5):272-277).

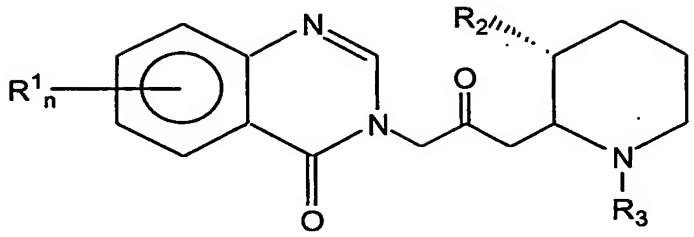
Intra-abdominal and retroperitoneal fibrosis have been described as secondary to intraperitoneal (IP) administration of several chemotherapeutic agents, including carboplatin , mitoxantrone and the combination of 5-fluorouracil and cisplatin (Fata et. al., Cancer 2000, Jun 1;88(11):2447-51).

Adriamycin, either primary or in liposomal formulations, is known to induce fibrotic encapsulation of tumors, with resultant decreasing concentrations in the tumor.

Halofuginone

US Patent 3,320,124, disclosed and claimed a method for treating coccidiosis with quinazolinone derivatives. Halofuginone, otherwise known as 7-bromo-6-chloro-3-[3-(3-hydroxy-2-piperidinyl)-2-oxopropyl]-4(3H)-quinazolinone (one of the quinazolinone derivatives), was first described and claimed in said patent by American Cyanamid company, and was the preferred compound taught by said patent and the one commercialized from among the derivatives described and claimed therein. Subsequently, US patents 4,824,847; 4,855,299; 4,861,758 and 5,215,993 all related to the coccidiocidal properties of Halofuginone.

More recently, it was disclosed in U.S. Patent No. 5,449,678 that these quinazolinone derivatives are unexpectedly useful for the treatment of a fibrotic condition. This disclosure provides compositions of a specific inhibitor comprising a therapeutically effective amount of a pharmaceutically active compound of the general formula I:



wherein: n=1-2

R_1 is at each occurrence independently selected from the group consisting of a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl. Pharmaceutically acceptable salts thereof are also included. Of this group of compounds, Halofuginone has been found to be particularly effective for such treatment.

U.S. Patent No. 5,449,678 discloses that these compounds are effective in the treatment of fibrotic conditions such as scleroderma and graft versus host disease (GVHD). U.S. Patent No. 5,891,879 further discloses that these compounds are effective in treating restenosis. The two former conditions are associated with excessive collagen deposition, which can be inhibited by Halofuginone. Restenosis is characterized by smooth muscle cell proliferation and extracellular matrix accumulation within the lumen of affected blood vessels in response to a vascular injury [Choi *et al.*, *Arch. Surg.*, **130**:257-261, 1995]. One hallmark of such smooth muscle cell proliferation is a phenotypic alteration, from the normal contractile phenotype to a synthetic one. Type I collagen

has been shown to support such a phenotypic alteration, which can be blocked by halofuginone [Choi *et al.*, *Arch. Surg.*, **130**: 257-261, 1995; U.S. Patent No. 5,449,678]. Notably, halofuginone inhibits collagen synthesis by fibroblasts in vitro, however, it promotes wound healing in vivo (WO 01/17531). Thus, the exact behavior of Halofuginone *in vivo* cannot always be accurately predicted from *in vitro* studies.

In addition quinazolinone containing pharmaceutical compositions including halofuginone have been disclosed and claimed as effective for treating malignancies (US 6,028,075) as well as for prevention of neovascularization (US 6,090,814).

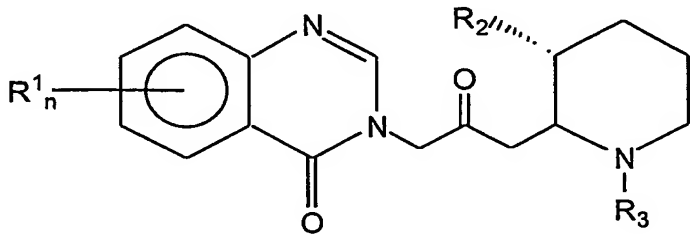
The ability of halofuginone, or other related quinazolinone derivatives to enhance the efficacy of known anti-tumor treatments including either radiation or chemotherapy by increasing the sensitivity of tumor cells to the toxic effect of these modalities or by other mechanisms, has not been taught or suggested in the prior art.

Thus, the ability of halofuginone, or other related quinazolinone derivatives to improve the efficacy of anti tumor treatments is both novel and non-obvious.

SUMMARY OF THE INVENTION

Unexpectedly, it has been found that pharmaceutical compositions containing quinazolinone derivatives, especially halofuginone, can improve the effectiveness of known anti tumor treatments, such as radiation and chemotherapy. It is postulated that the synergistic effect of the quinazolinone is mediated by increasing the sensitivity of tumor cells to the cytotoxic effects of ionizing radiation or chemotherapy treatment, or by engendering an enhanced sensitivity to immune effects, although other mechanisms can also be responsible.

According to the present invention, there is provided a composition for increasing the effectiveness of known anti tumor treatments, comprising a quinazolinone derivative compound having the general formula I:



wherein: n=1-2

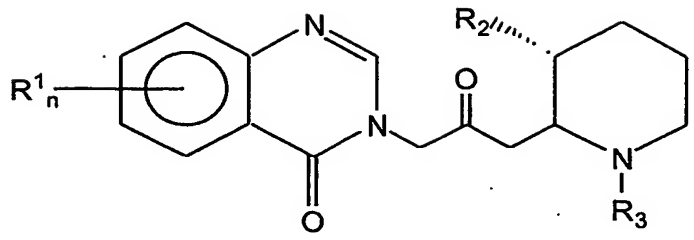
R_1 is a at each occurrence independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, and pharmaceutically acceptable salts thereof;

together with a known anti tumor treatment.

According to another embodiment of the present invention, there is provided a method for increasing the effectiveness of known anti-tumor treatments, by co-administering quinazolinone derivative compounds having the formula:



wherein: n=1-2

R_1 at each occurrence is independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

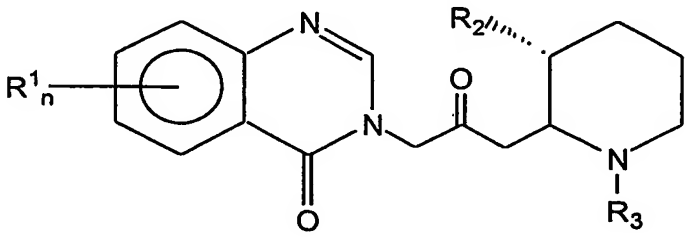
R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, and pharmaceutically acceptable salts thereof; combined with a known anti-tumor treatment.

The method or co-treatment is particularly effective when the known cancer therapy is selected from the group consisting of radiation therapy and chemotherapy.

According to another more preferred embodiment the co-treatment is not performed with a mixture of the two agents but rather performed in separate administrations of each of the agents, namely the quinazolinone and the known anti-tumor agent.

According to another embodiment of the present invention, there is provided a method for increasing the effectiveness of known anti-tumor treatments, by pre-administering quinazolinone derivative compounds having the formula:



wherein: n=1-2

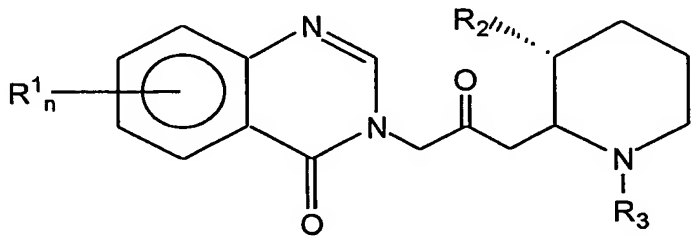
R_1 at each occurrence is independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, and pharmaceutically acceptable salts thereof; followed by a known anti-tumor treatment.

The method of pretreatment is particularly effective when the known cancer therapy is selected from the group consisting of radiation therapy and chemotherapy

Another embodiment of the present invention, is the use of a quinazolinone derivative of the general formula I:



wherein: n=1-2

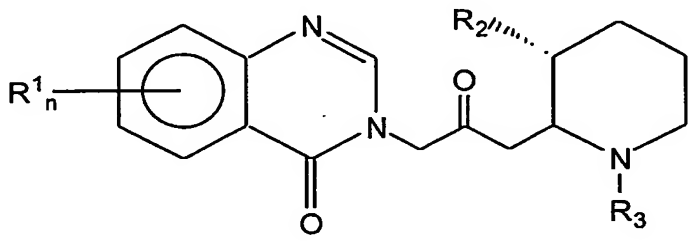
R_1 at each occurrence is independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, pharmaceutically acceptable salts thereof, in preparation of a pharmaceutical composition for increasing the effectiveness of known anti tumor treatments, wherein

the quinazolinone derivative compounds having the formula I are combined with any anti tumor treatment.

According to another embodiment of the present invention, there is provided a method for alleviating or preventing the damage induced by radiation therapy, by using quinazolinone derivative compounds having the formula:



wherein: n=1-2

R_1 at each occurrence is independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, and pharmaceutically acceptable salts thereof.

Treatment with quinazolinones according to the present invention can be particularly effective and beneficial when administered preceding administration of a known anti-tumor chemotherapeutic agent or treatment with radiation therapy. This advantage is attained by the use of halofuginone to synchronize cells thereby rendering them more susceptible to the subsequent anti-tumor treatment.

Nevertheless, co-administration of the two agents, whether as a single combined composition or in separate compositions is also shown to act synergistically.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1-Halofuginone arrest – rabbit aortic SMC cell cycle in late G1 phase.

Fig. 2- Leg contraction as a model for Radiation induced fibrosis.

Fig. 3 -Daily I.P. injection of Halofuginone (μg/mouse) –post leg Irradiation.

Fig. 4- Radiation survival curves for two human pancreatic cancer cell lines pre-treated with Halofuginone.

DETAILED DESCRIPTION OF THE INVENTION

The combination of different modalities in cancer treatment is gaining a lot of enthusiasm. Combining different modalities or even specific agents with different mechanism of action and different adverse effects, allows for better efficacy with fewer side effects.

In this context quinazolinone derivatives, preferably halofuginone, may improve the effect of other anti-tumor agents or treatments either through enhancing the anti-tumor effect or through lessening adverse effects.

Unexpectedly, it has been found, as exemplified in detail hereinbelow, that pharmaceutical compositions containing quinazolinone derivatives, preferably halofuginone, can synergistically enhance the effectiveness of anti-tumor treatments which are known, including but not limited to radiation therapy and chemotherapy.

The mechanism of enhancing the sensitivity to chemotherapy or irradiation is not clear, and possibly acts by increasing the sensitivity of tumor cells to the toxic effects of ionizing radiation or chemotherapy treatment, although other mechanisms can also be involved.

Some of the most effective and commonly used chemotherapy agents including but not limited to taxol, gemacetabin, vinca alkaloids and many others, are known to affect cancer cells in a specific stage of the cell cycle. These agents may therefore be described as "cell cycle specific agents". The cell cycle can be described as a sequence of phases through which the cell proceeds as it proliferates. The phases of this cycle are denoted G1, S, G2 and M, where G1 is the gap preceding synthesis of DNA, S is the phase during which the cell synthesizes DNA, G2 is the gap between the S phase and division or mitosis (M). Cells that are not proliferating may be arrested in a stage referred to as G_0.

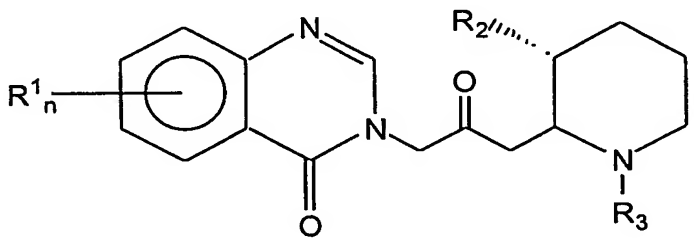
It was shown that halofuginone reversibly arrests cells in the G0/G1 stage. Upon removal of halofuginone cells are able to enter the S phase and continue cycling (Nagler et. al. Kidney Int. Vol. 52(1997), pp.1561-1569). Therefore, the combination of halofuginone as a synchronizing agent will sensitize the tumor cells to a bolus of a cell cycle dependent agent, as defined above. Upon exposure to halofuginone the cell cycle will be arrested, whereas upon removal of the compound, the cancer cells will regain their normal cycling. Effectively, this serves to synchronize the cells, thus bringing a larger proportion of the cells to the specific stage of the cell cycle where they will be sensitive to the effects of the chemotherapeutic agent.

Specific non-limiting examples of suitable drugs include cancer chemotherapeutic agents as exemplified by the compounds doxorubicin, daunorubicin, idarubicin, epirubicin, melphalan, dacarbazine, cisplatinum, carboplatin and mithomycin.

Additional cancer chemotherapeutic agents suitable for use in conjunction with the compositions and methods of the present invention may be selected from the following categories:

topoisomerase inhibitors; spindle poison vincas: vinblastine, vincristine, vinorelbine, paclitaxel (taxol), docetaxel; alkylating agents: mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide; methotrexate; anti-metabolites: 6-mercaptopurine, 5-fluorouracil, cytarabine, gemcitabin; podophyllotoxins: etoposide, irinotecan, topotecan; antibiotics: doxorubicin (adriamycin), bleomycin, mitomycin; nitrosoureas: carmustine (BCNU), lomustine; inorganic ions: cisplatin, carboplatin, oxaloplatin; interferon, asparaginase; hormones: tamoxifen, leuprolide, flutamide, megestrol acetate

According to the present invention, there is provided a composition for increasing the effectiveness of known anti-tumor treatments, including but not limited to radiation and chemotherapy, by using Quinazolinone derivative compounds having the formula:



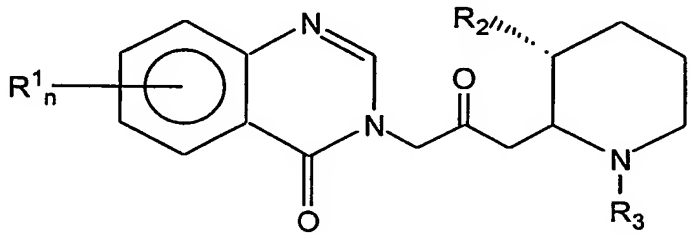
wherein: n=1-2

R_1 is at each occurrence independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, as well as pharmaceutically acceptable salts thereof, combined with any anti tumor treatment.

According to another embodiment of the present invention, there is provided a method for increasing the effectiveness of known anti tumor treatments, including but not limited to radiation and chemotherapy, by using Quinazolinone derivative compounds having the formula:



wherein: n=1-2

R_1 is at each occurrence independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl.

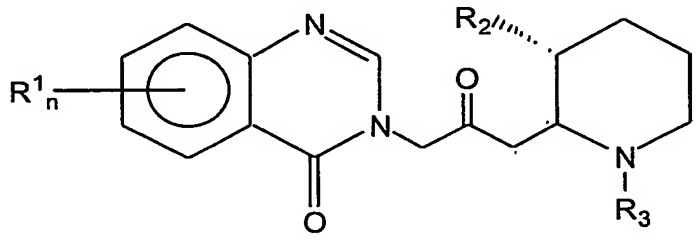
pharmaceutically acceptable salts thereof are also included, combined with any anti tumor treatment.

According to a currently preferred embodiment of the present invention, the enhancement of the effectiveness of known anti-tumor treatments is obtained by pretreatment with a quinazolinone of general formula I, preferably halofuginone. This is particularly effective when the additional anti-cancer treatment is radiation therapy.

According to another currently preferred embodiment of the present invention, the enhancement of the effectiveness of known anti-tumor treatments is obtained by

treatment with a quinazolinone of general formula I, preferably halofuginone, at substantially the same time as the treatment with the additional known anti-cancer agent. Administration may be in a single composition or in separate compositions as appropriate for the optimal formulation of each agent.

Another embodiment of the present invention, is use of Quinazolinone derivative compounds in preparing a pharmaceutical composition for increasing the effectiveness of known anti tumor treatments, including but not limited to radiation and chemotherapy, wherein the Quinazolinone derivative compounds having the formula:

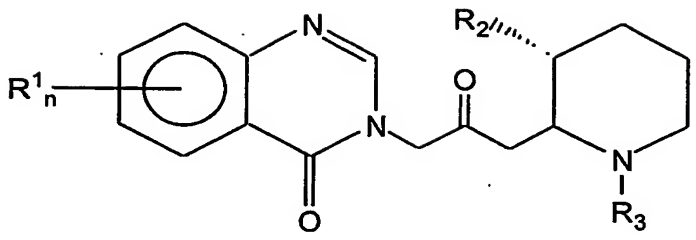


wherein: n=1-2

R_1 is at each occurrence independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, and pharmaceutically acceptable salts thereof, combined with a known anti tumor treatment.

According to another embodiment of the present invention, there is provided a method for alleviating or preventing the damage induced by radiation therapy, by using Quinazolinone derivative compounds having the formula:



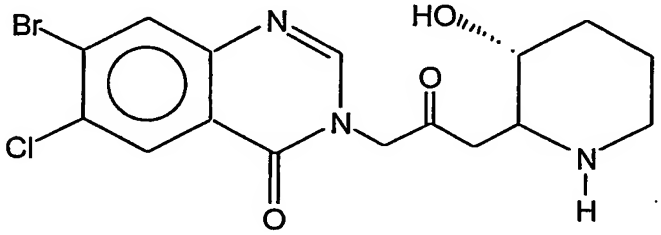
wherein: n=1-2

R_1 which at each occurrence is independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and

R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, and pharmaceutically acceptable salts thereof.

According to further preferred embodiments of the present invention, the compound is preferably Halofuginone. Hereinafter, the term "Halofuginone" is defined as a compound having the formula:



and pharmaceutically acceptable salts thereof. The composition preferably further comprises a pharmaceutically acceptable carrier for the compound.

The anti tumor treatments in the present invention include radiation therapy, chemotherapy, immunotherapy, hormonal therapy and genetic therapy.

Currently more preferred compounds for chemotherapeutic treatment in conjunction with the quinazolinone compositions of the invention are selected from the groups consisting of topoisomerase inhibitors, spindle poison vincas: vinblastine, vincristine, vinorelbine (taxol), paclitaxel, docetaxel; alkylating agents: mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide; methotrexate; 6-mercaptopurine; 5-fluorouracil, cytarabine, gemcitabin; podophyllotoxins: etoposide, irinotecan, topotecan; antibiotics: doxorubicin (adriamycin), bleomycin, mitomycin; nitrosoureas: carmustine (BCNU), lomustine; inorganic ions: cisplatin, carboplatin; interferon, asparaginase; hormones: tamoxifen, leuprolide, flutamide, megestrol acetate.

Hereinafter, the term "subject" refers to the human or animal to whom halofuginone is administered. The term "anti tumor treatments" refers to any anti tumor treatment approved for use in a subject. The term "Radiation therapy" refers to treatment of cancer through ionizing radiation, as is well known in the art. The term "chemotherapy" refers to treatment of a disease characterized by abnormal cell proliferation with chemicals or drugs. The term "immunotherapy" refers to treatment of disease by modulation of the Immune system and/or responses. The term "hormonal therapy" refers to treatment of disease characterized by abnormal cell proliferation with different hormones or their inhibitors. The term "genetic therapy " refers to treatment of disease characterized by abnormal cell proliferation with compositions containing different genes or gene products, including antisense therapy.

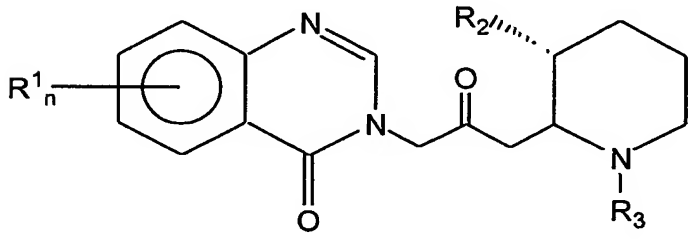
Hereinafter, the term "oral administration" includes, but is not limited to administration by mouth for absorption through the gastrointestinal tract, buccal administration and sublingual administration.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents, flavorings, dispersing aids, emulsifiers, binders or preservatives may be desirable.

The term "parenteral administration" includes, but is not limited to, administration by intravenous drip or bolus injection, subcutaneous, intramuscular injection or direct injection into or adjacent to the tumor.

Formulations for parenteral administration may include but are not limited to sterile aqueous solutions, which may also contain buffers, diluents and other suitable additives.

Although the specific quinazolinone derivative "halofuginone" is referred to throughout the specification, it is understood that other quinazolinone derivatives may be used in its place, these derivatives having the general formula I:



wherein: n=1-2

R_1 is at each occurrence independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl, and pharmaceutically acceptable salts thereof.

Compounds, which are intended for the enhancing the anti tumor treatments must be tested in an *in vivo* model for their ability.

Such experiments were conducted for Halofuginone, as described in greater detail in the Examples below.

While the invention will now be described in connection with certain preferred embodiments in the following figures and examples so that aspects thereof may be more fully understood and appreciated, it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus, the following figures and examples which include preferred embodiments will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of formulation procedures as well as of the principles and conceptual aspects of the invention.

EXAMPLES

EXAMPLE 1

Halofuginone was tested effect in conjunction with BCNU (golden standard for Brain Gliomas) treatment was evaluated.

Halofuginone was tested in the human T98G glioblastoma xenograft implanted subcutaneously or intracranially. Mice were implanted with the human T98G glioblastoma tumor cells subcutaneously in a thigh or intracranially.

The endpoint for the subcutaneous tumor was tumor growth delay while the endpoint for the intracarnial tumor was increase-in-lifespan (survival).

Halofuginone was administered orally by gavage at dose levels of 0.1,0.2, and 0.5 mg/kg/day, once per day, on days 4 through 34 days post tumor implantation.

The experiment was terminated on day 151 post tumor implantation.

There was no effect of halofuginone on the body weight of the animals.

Table 1

Response of the Human T98G Glioblastoma Multiforme to treatment withBCNU alone or along with Halofuginone		
TREATMENT GROUP	**TUMOR GROUTH DELAY, Days (sc tumor)**	**SURVIVAL, Days (ic tumor)**
CONTROLS	0	64 ± 10
BCNU (15mg/kg)ip,d7,9,11	3.9 ± 0.4	97 ± 21
BCNU + 0.1 mg/kg		
BCNU + 0.2 mg/kg	6.4 ± 0.8	103 ± 29
BCNU + 0.5 mg/kg	11.1 ± 1.0	148 ± 4
	12.2 ± 1.6	119 ± 21

- The experiment was terminated on day 151.
- There were five animals per group.

Combination of Halofuginone with BCNU elevated dramatically the tumor growth delay from 3.8 days to 12 days.

In the intracranial model Halofuginone in combination with BCNU significantly increased the life span of mice compared to BCNU alone. The pharmacological optimal dose of 0.2mg/kg BW prolonged the survival beyond the scope of the study (animals sacrificed in good health after 150 days).

Both effects were dose dependent.

EXAMPLE 2

Halofuginone cell cycle arrest - rabbit aortic smooth muscle cells (SMC) cell cycle arrest in late G1 phase.

Experiments were done to determine where in the cell cycle Halofuginone inhibited cell growth.

As determined by [^{3}H]-thymidine incorporation, addition of 10% FBS to growth-arrested, quiescent SMC promotes entry to S phase after a G_1 period of 16 hours and maximal DNA synthesis was seen 20 hours after serum-stimulation (Fig. 1).

When Halofuginone (10^{-7} M) was added with 10% FBS, only low levels of [^{3}H]-thymidine incorporation were observed (Fig. 1). We next determined if Halofuginone arrested proliferation at a specific point in the cell cycle. For these experiments, quiescent SMCs were kept in 10% FBS plus 10^{-7} M Halofuginone for 24 hours. The cultures were then washed and placed in 10% FBS with [^{3}H]-thymidine and without Halofuginone. At various times after Halofuginone removal, the cells were harvested and thymidine incorporation was determined. When Halofuginone-treated cells were released from growth-arrest, there was a lag of 4-6 hours before initiation of DNA synthesis, which peaked by 10 hours (Fig. 1).

Since quiescent G_0-arrested SMCs require a minimum of about 16 hours to pass through G_0, pretreatment with 10% FBS plus Halofuginone permitted cell cycle progression to a point about 4-6 hours from S phase. Thus, in the continual presence of Halofuginone, SMC progress into G_1 and reversibly arrest at late G_1 phase.

EXAMPLE 3

Halofuginone cell cycle arrest Rat mesangial cells (RMC) –cell cycle arrest in G_0/G_1 phase

We have determined if halofuginone arrested mesangial cell proliferation at a specific stage of the cell cycle.

For this purpose sub confluent RMC's were kept in 10% FCS in the absence or presence of 150 ng/ml Halofuginone for 24 hours. The cells were then harvested,

stained with propidium iodide and analyzed by FACScan. The percentage of cells progressing into G_2/M phase was reduced by Halofuginone from 20% to 7%, while the percentage of cells in G_0/G_1 was increased from 38% to 65% in the absence and presence of Halofuginone, respectively. These results indicate that in the presence of Halofuginone, a large proportion of the MCs are arrested in the G_0/G_1 phase.

EXAMPLE 4

Halofuginone decreased fibrosis induced by radiation

Mice were intraperitoneally injected with 1-5 μg/mouse of Halofuginone once daily, for 4 months.

Only the right leg of each animal was radiated with 35Gy or 45Gy. In the control group mice did not receive Halofuginone and the right leg was radiated with 35Gy or 45Gy. Within the time periods of 2 to 4 months after radiation, leg contraction was measured, as demonstrated in Fig. 2.

As is seen in Fig. 3, in the Halofuginone treated animals there is a dramatic decrease in the "leg length difference" between the right and left leg. The effect of Halofuginone can be observed post radiation at 2 and 4 months, at 35Gy and 45 Gy radiation and at all of the used Halofuginone concentrations (1-5μg/mouse).

In general the irradiated leg in the Halofuginone treated mice is definitely less stiff in comparison to the untreated one and the skin is less dry.

Thus Halofuginone reduced the effects of radiation in this model.

EXAMPLE 5

Halofuginone acts as a radiation sensitizer

Two pancreatic cancer cell lines : 3602 xrt and 3602 Zyrd/Xrt were incubated with or without 250nM Halofuginone for 24hr, than followed with 0-8 Gy.

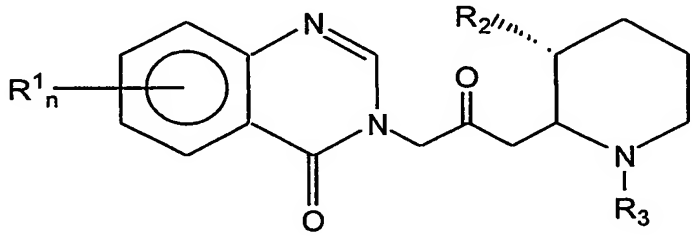
Survival fraction of the cell was determined (Fig. 4).

Halofuginone caused a decrease in the survival fraction of approximately 50%.

The forgoing examples are meant to be construed as non-limitative in nature. It will be readily apparent to the skilled practitioner that many variations, additions, substitutions and modifications thereof are possible within the scope of the invention, which is to be limited by the claims that follow.

CLAIMS:

1. A method for improving the effectiveness of an anti-tumor treatment by administration to a subject in need thereof of a pharmaceutical composition containing as an active ingredient a quinazolinone derivative compound having the general formula I:



wherein: n=1-2

R_1 at each occurrence is independently selected from the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl or pharmaceutically acceptable salts thereof, with at least one pharmaceutically acceptable carrier or diluent,

together with a known anti tumor treatment.

2. The method according to claim 1 wherein the subject is human.

3. The method of claim 1 wherein the administration is prior to the anti-tumor treatment.

4. The method of claim 1 wherein the administration is substantially at the time of the known anti-tumor treatment.

5. The method of claim 4 wherein the co-administration is in a single pharmaceutical composition.

6. The method of claim 4 wherein the co-administration is in separate pharmaceutical compositions.

7. The method according to claims 1-6 wherein the anti tumor treatment is radiation therapy.

8. The method according to claims 1-6 wherein the anti tumor treatment is chemotherapy.

9. The method according to claims 1-6 wherein the anti tumor treatment is immunotherapy.

10. The method according to claims 1-6 wherein the anti tumor treatment is hormonal therapy.

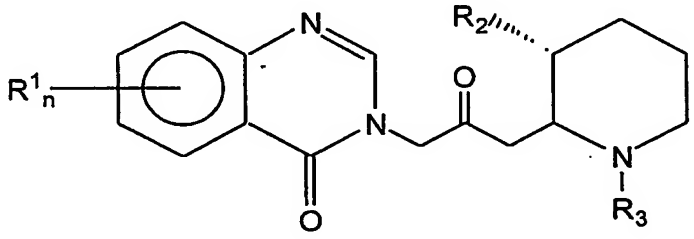
11. The method according to claims 1-6 wherein the anti tumor treatment is genetic therapy.

12. The method according to any previous claims wherein the improvement is achieved by enhancement of cellular sensitivity to the anti tumor treatments.

13. The method according to any one of claims 1-12 wherein the compound is halofuginone or a halofuginone derivative.

14. The method according to claim 8, wherein the compound used for chemotherapy is a topoisomerase inhibitor, spindle poison vincas: vinblastine, vincristine, vinorelbine, paclitaxel (taxol), docetaxel; an alkylating agent: mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide; methotrexate; antimetabolites: 6-mercaptopurine; 5-fluorouracil, cytarabine, gemcitabin; podophyllotoxins: etoposide, irinotecan, topotecan; antibiotics: doxorubicin (adriamycin), bleomycin, mitomycin; nitrosoureas: carmustine (BCNU), lomustine; inorganic ions: cisplatin, carboplatin, oxaloplatin; interferon, asparaginase; hormones: tamoxifen, leuprolide, flutamide, megestrol acetate .

15. A pharmaceutical composition containing as an active agent ingredient a quinazolinone derivative compound having the general formula I:



wherein: n=1-2

R_1 at each occurrence is independently selected from the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl or pharmaceutically acceptable salts thereof, and at least one pharmaceutically acceptable carrier or diluent, further comprising a known anti tumor agent, thereby improving the effectiveness of the anti tumor treatment.

16. The pharmaceutical composition according to claim 15 wherein the anti tumor treatment is chemotherapy.

17. The pharmaceutical composition according to claim 15 wherein the anti tumor treatment is immunotherapy.

18. The pharmaceutical composition according to claim 15 wherein the anti tumor treatment is hormonal therapy.

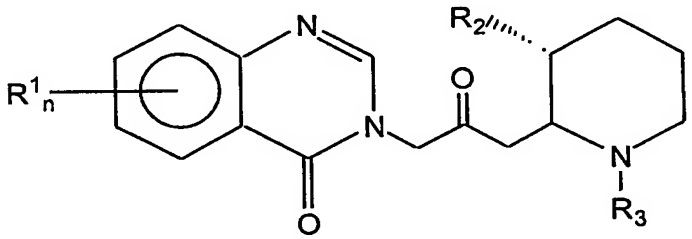
19. The pharmaceutical composition according to claim 15 wherein the anti tumor treatment is genetic therapy.

20. The pharmaceutical composition according to any one of claims 15-19 wherein the improvement is achieved by enhancement of cellular sensitivity to the anti tumor treatments.

21. The pharmaceutical composition according to claims 15-20 wherein the compound of Formula (I) is halofuginone or a halofuginone derivative.

22. The pharmaceutical composition according to claims 16, wherein the compound used for chemotherapy is a topoisomerase inhibitor, spindle poison vincas: vinblastine, vincristine, vinorelbine, paclitaxel (taxol), docetaxel; an alkylating agent: mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide; methotrexate; anti-metabolites: 6-mercaptopurine; 5-fluorouracil, cytarabine, gemcitabin; podophyllotoxins: etoposide, irinotecan, topotecan; antibiotics: doxorubicin (adriamycin), bleomycin, mitomycin; nitrosoureas: carmustine (BCNU), lomustine; inorganic ions: cisplatin, carboplatin, oxaloplatin; interferon, asparaginase; hormone agonist or antagonists: tamoxifen, leuprolide, flutamide, megestrol acetate .

23. Use of a quinazolinone derivative compound having the general formula I:



wherein: n=1-2

R_1 at each occurrence is independently selected from the group consisting of the hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl or pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier or

diluent in preparation of a composition for administration to a subject combined with a known anti tumor treatment, for improving the effectiveness of the anti tumor treatment.

24. Use according to claim 23, wherein the subject is human.

25. Use according to claim 23, wherein the anti tumor treatment is radiation biology.

26. Use according to claim 23, wherein the anti tumor treatment is chemotherapy.

27. Use according to claim 23, wherein the anti tumor treatment is immunotherapy.

28. Use according to claim 23, wherein the anti tumor treatment is hormonal therapy.

29. Use according to claim 23, wherein the anti tumor treatment is genetic therapy.

30. Use according to claims 23-29, wherein the improvement is achieved by enhancement of cellular sensitivity to the anti tumor treatments.

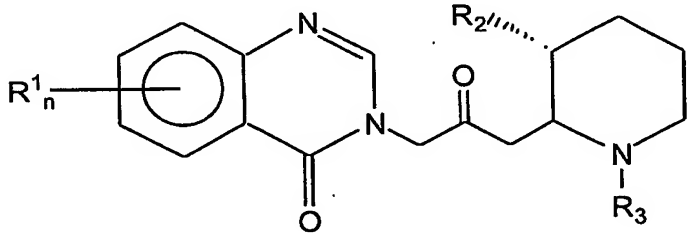
31. Use according to claims 25-30 wherein the compound is Halofuginone or a Halofuginone derivative.

32. Use according to claim 26, wherein the compound used for chemotherapy is a topoisomerase inhibitor, spindle poison vincas: vinblastine, vincristine, vinorelbine, paclitaxel (taxol), docetaxel; an alkylating agent: mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide; methotrexate; anti-metabolites: 6-

mercaptopurine; 5-fluorouracil, cytarabine, gemcitabin; podophyllotoxins: etoposide, irinotecan, topotecan; antibiotics: doxorubicin (adriamycin), bleomycin, mitomycin; nitrosoureas: carmustine (BCNU), lomustine; inorganic ions: cisplatin, carboplatin, oxaloplatin; interferon, asparaginase; hormone agonists or antagonists: tamoxifen, leuprolide, flutamide, megestrol acetate .

33. The pharmaceutical composition of any one of claims 15-22 wherein said pharmaceutically acceptable carrier is suitable for administration of the composition orally or parenterally in the form of powder, granules, suspensions or solutions in water or non aqueous media, sachets, capsules or tablets.

34. A method for alleviating or preventing the damage induced by anti tumor treatments, by administration of a pharmaceutical composition containing as an active ingredient a quinazolinone derivative compound having the formula I:



wherein: n=1-2

R_1 at each occurrence is independently a member of the group consisting of hydrogen, halogen, nitro, benzo, lower alkyl, phenyl and lower alkoxy;

R_2 is a member of the group consisting of hydroxy, acetoxy and lower alkoxy; and R_3 is a member of the group consisting of hydrogen and lower alkenoxy-carbonyl or

pharmaceutically acceptable salts thereof, with pharmaceutically acceptable carriers, to a subject.

35. The method of claim 34 wherein the administration is prior to the anti-tumor treatment.

36. The method of claim 35 wherein the administration is substantially at the time of the known anti-tumor treatment.

37. The method of claim 34-36 wherein the co-administration is in a single pharmaceutical composition.

38. The method of claim 36-38 wherein the co-administration is in separate pharmaceutical compositions.

39. The method according to claims 34-38 wherein the anti tumor treatment is radiation therapy.

40. The method according to claims 34-38 wherein the anti tumor treatment is chemotherapy.

41. The method according to claim 34-38 wherein the anti tumor treatment is immunotherapy.

42. The method according to claim 34-38 wherein the anti tumor treatment is hormonal therapy.

43. The method according to claim 34-38 wherein the anti tumor treatment is genetic therapy.

44. The method according to claims 34-38 wherein the compound is halofuginone or a halofuginone derivative.

45. The method according to claim 40, wherein the compound used for chemotherapy is selected from the group consisting of a topoisomerase inhibitor, spindle poison vincas: vinblastine, vincristine, vinorelbine, paclitaxel (taxol), docetaxel; an alkylating agent: mechlorethamine, chlorambucil, cyclophosphamide, melphalan, ifosfamide; methotrexate; anti-metabolites: 6-mercaptopurine; 5-fluorouracil, cytarabine, gemcitabin; podophyllotoxins: etoposide, irinotecan, topotecan; antibiotics: doxorubicin (adriamycin), bleomycin, mitomycin; nitrosoureas: carmustine (BCNU), lomustine; inorganic ions: cisplatin, carboplatin, oxaloplatin; interferon, asparaginase; hormone agonists or antagonists: tamoxifen, leuprolide, flutamide, megestrol acetate.

For the applicants

Cynthia Webb

Patent Attorney

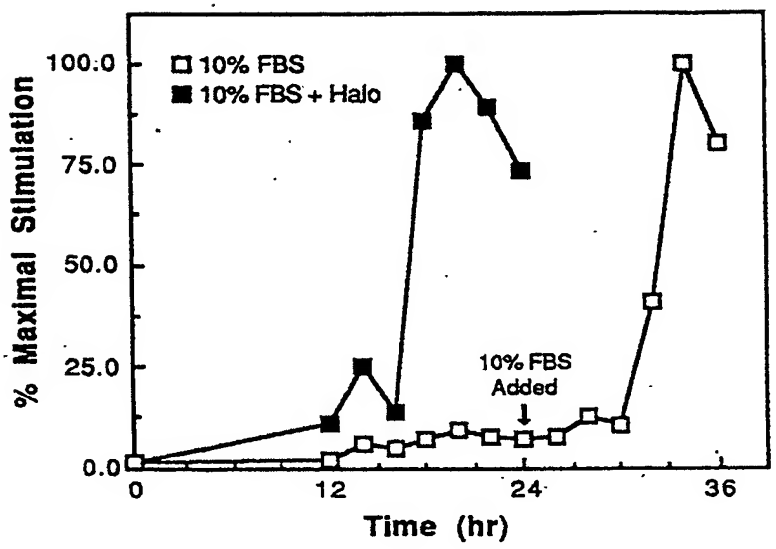


Fig. 1

Halofuginone arrest – rabbit SMC cell cycle in late G1 phase

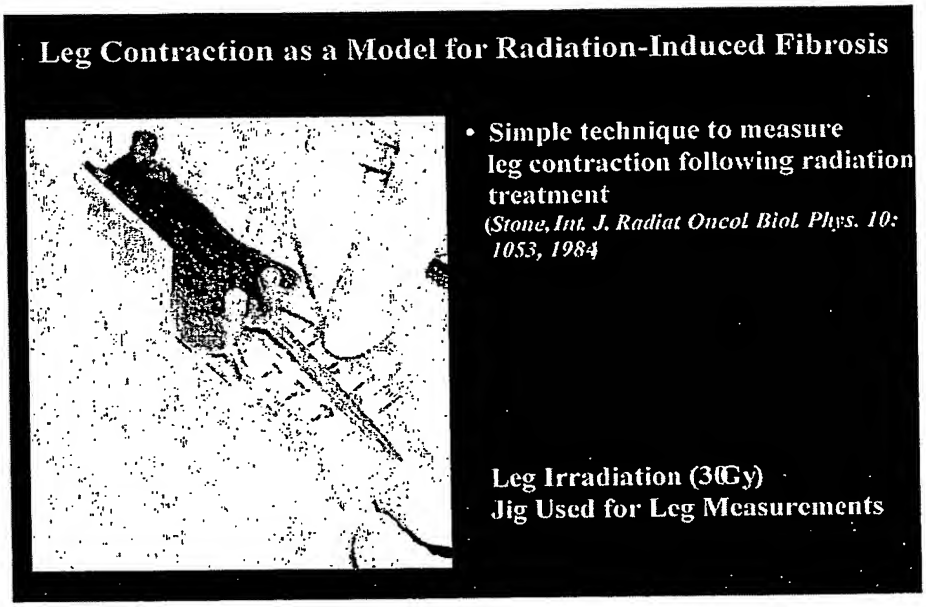

Leg Contraction as a Model for Radiation-Induced Fibrosis
• Simple technique to measure leg contraction following radiation treatment
(Stone, Int. J. Radiat Oncol Biol Phys. 10: 1053, 1984
Leg Irradiation (30Gy)
Jig Used for Leg Measurements

Fig. 2

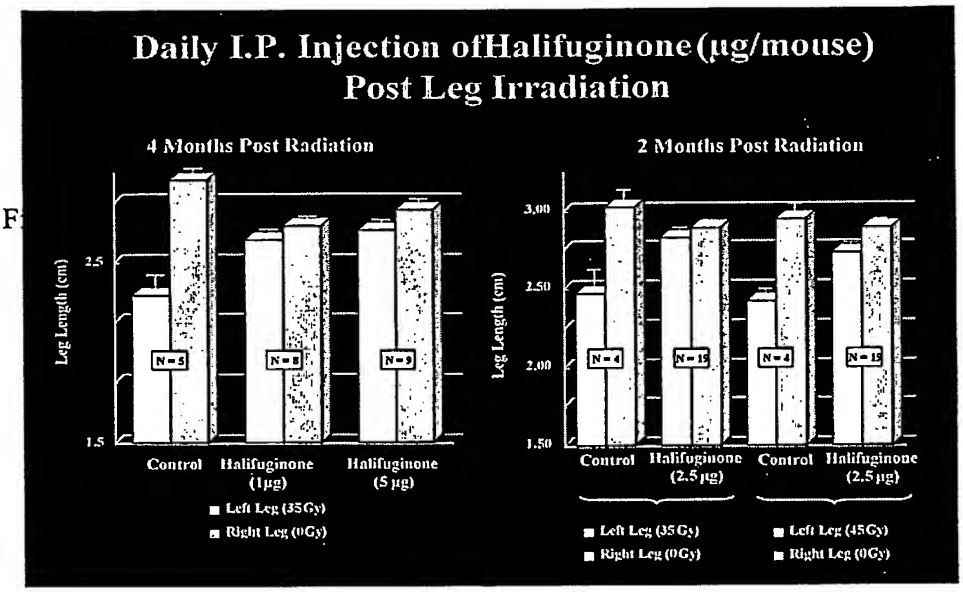

Daily I.P. Injection ofHalifuginone(µg/mouse)
Post Leg Irradiation
4 Months Post Radiation
2 Months Post Radiation
Leg Length (cm)
2.5
1.5
3.00
2.50
2.00
1.50
N=5
N=8
N=9
N=4
N=15
N=4
N=15
Control
Halifuginone (1µg)
Halifuginone (5 µg)
Control
Halifuginone (2.5µg)
Control
Halifuginone (2.5 µg)
Left Leg (35Gy)
Right Leg (0Gy)
Left Leg (35Gy)
Right Leg (0Gy)
Left Leg (45Gy)
Right Leg (0Gy)

Fig. 3

Fig. 4

Radiation survival curves for two human pancreatic cancer cell lines pre-treated with Halofuginone.